CONCERNING COMPENSATORY HYPERPLASIA OF THE INTIMA.[1]

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Plate LXIX.

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In 1885 Thoma set forth his views concerning the fundamental principles underlying arteriosclerosis. From the first Thoma's attention was concentrated on the intimal thickening which occurred in various arteries, and more particularly upon those developing in the aorta. His studies of these lesions were chiefly concerned with the general characters of the disease, to some extent with the nature of the tissues involved, and occasionally with the degenerative processes which develop in these intimal thickenings. According to his observations the intimal thickenings had common characteristics in their development, but only two types of the disease were recognized. In one instance the intimal thickening was diffuse and uniform, sometimes encircling the entire artery; in other cases nodular thickenings were evident, which might or might not be associated with the openings of smaller arteries. In his opinion the intimal thickening of both types developed in a process of connective tissue hyperplasia which was associated with a widening of the vessel lumen.

Thoma had previously convinced himself that shortly after birth there was an increase in the connective tissue of the intima of the aorta, extending from the mouth of the ductus arteriosus to and into the hypogastric arteries. His explanation for this development of connective tissue was that the loss of the placental circulation at birth led to the disproportion of the blood content to the vessel wall; that is, he believed that after birth the amount of blood in the vessels forming the foetal circulation was less than was

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present during intra-uterine life, and hence, to compensate for this diminished amount of blood, a new layer of connective tissue developed in the lumen, in order to make the tube narrower.

Although it has been found that there is a gradual development of intimal tissues with progressive age, no association can be demonstrated between the loss of placental circulation and the tissue hyperplasia. Nor can we support Thoma's findings that this new tissue in the intima is confined alone along the path of the fetal circulation. Moreover, the hyperplasia which occurs in the intima often involves elements other than connective tissue. Furthermore, the principle of compensation between the fluid of the body and the fixed tissues is usually balanced by other means. Under ordinary conditions the diminished amount of blood in the vascular system is readily compensated by the absorption of fluid from the surrounding tissues. How frequently during life must there be a temporary disproportion of the fluid blood to the arteries! Severe hemorrhages following trauma, hemorrhages occurring in typhoid and dysentery, and loss of blood occurring in cases of hemophilia all lead to this disproportion of blood content to the vascular system, and yet in these cases the disproportion is but temporary and the proportion can be easily reëstablished by the absorption of fluid. Moreover, in none of these diseases do we see a process of compensatory hyperplasia such as is described by Thoma.

This general principle of compensatory hyperplasia, which Thoma claimed for the connective tissue growth in the intima of the aorta of children, was also adapted by him for the explanation of the intimal thickening occurring in later life. Thoma discusses the subject in a general way only, and he avoids minute histological descriptions.

In 1886 Thoma carried out a series of experiments to demonstrate compensatory hyperplasia. He filled the fresh aorta in the cadaver, or aortæ recently taken from the cadaver, with melted paraffin under a pressure about equal to the blood pressure in the aorta. These injected preparations were kept at this internal pressure until the paraffin had thoroughly hardened. The aorta was then split open and the paraffin core removed. On examining these wax models Thoma claimed that the surface was uniformly smooth,

and that opposite the sites of nodular sclerosis the paraffin showed no indentation such as might be expected from localized intimal thickenings.

Moreover, on examining the arterial wall, which had been subjected to the continuous pressure of the paraffin, he found that the intimal surface was quite smooth and free from the endarteritic irregularities such as are found in the collapsed arteries at post-mortem.

Thoma further examined cross-sections of these arteries and found that not infrequently the media was thinned opposite the endarteritic plaque. He stated also, elsewhere, that the thickened intima might overlie an area of the media which appeared normal or even hypertrophied.

From his various observations Thoma deduced his well-known theory, the main features of which are briefly these: (1) the beginning of an arteriosclerosis, either endarteritis nodosa or diffusa, results from a weakening of the media which allows the artery to dilate either over a small circumscribed area or one involving a considerable portion of the arterial wall; (2) this dilatation of the arterial wall causes a widening of the lumen, which in turn induces a slowing of the blood current at this point. This slowing of the blood stream now acts as a local irritant leading to his so-called "compensatory hypertrophy of the intima."

Thoma's theories to-day are upheld by some and denounced by others. As we have improved our technique for pathological examination, it has been shown that some of his views are untenable.

During the foregoing winter, 1909, I repeated some of Thoma's experiments, but had little success in the use of paraffin as an injection fluid. I found that paraffin was liable to produce artefacts which were misleading. When paraffin was injected into the aorta, the globules of water adhering to the fresh vessel wall produced irregularities in the mold of the hardened wax. I also found that the paraffin, after it had set and when the pressure of the fluid paraffin without had no effect upon it, underwent a considerable shrinkage, producing other artefacts, and in my specimens it always showed extensive irregularities of the intima on the paraffin core. In no instance could I substantiate Thoma's claim that the lumen

of an endarteritic artery was smooth and regular when under pressure.

However, remembering that there was considerable shrinkage in the paraffin while hardening, the possibility remained that when the vessel was properly distended at normal pressure or nearly so, the vessel wall was smooth, even with the presence of endarteritic plaques. It was, therefore, decided to use water for the injection fluid, and to fix the preparation by freezing. An apparatus was then devised so as to give any desired pressure, depending upon the height of the column of water above the aorta to be tested. When the proper pressure, equal to one hundred and sixty millimeters of mercury, had been obtained in the aorta, the whole apparatus was placed at a temperature of from 0° F. to —20° F. After an exposure of some hours the water in the specimen and apparatus was completely frozen and the character of the lumen was determined by sawing the frozen aorta into a series of small discs half an inch in thickness. The character of the lumen was easily distinguished in these discs, and impressions of the vessel wall in the distended state with the ice within were easily obtained. All the observations had, of course, to be made in the cold.

It was found that when the discs had been painted over with methylene blue and the excess stain had been removed with blotting paper, that accurate impression of the contour of the vessel wall could be obtained. Moreover, these impressions indicated with accuracy the contour of the lumen of the vessel while still under pressure. It is to be remembered that, as our specimens were filled with water under average high arterial pressure, the process of freezing, instead of relieving this pressure to some extent as is the case in the use of paraffin, actually increases the pressure, and hence would tend to obliterate still further the intimal projections and favor the views of Thoma.

In these experiments we were again unable to substantiate Thoma's contention. The intima with its white endarteritic thickenings could be quite readily distinguished from the media. Each of these endarteritic plaques formed a rounded bulging into the vessel lumen and nowhere could any weakening of the media be distinguished in the early intimal lesions. The small superficial

FIG. 1.

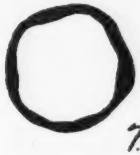

FIG. 2.

FIG. 1. Abdominal aorta of man aged 63, with extensive arterio-sclerosis and chronic endarteritis. Lumen much distorted.

FIG. 2. Aorta of man aged 75. Nos. 1, 2 and 3 are from thoracic aorta; Nos. 4 to 8 are from abdominal aorta. Chronic endarteritis with deep atheroma. Intimal lesions encroach into lumen. Media thinned in places.

fatty plaques which occur about the intercostal vessels were observed to rest upon the inner surface of the intima, like small masses of excess tissue. In many instances neither the deep intima nor the media showed any change in their structure or contour (Fig. 5).

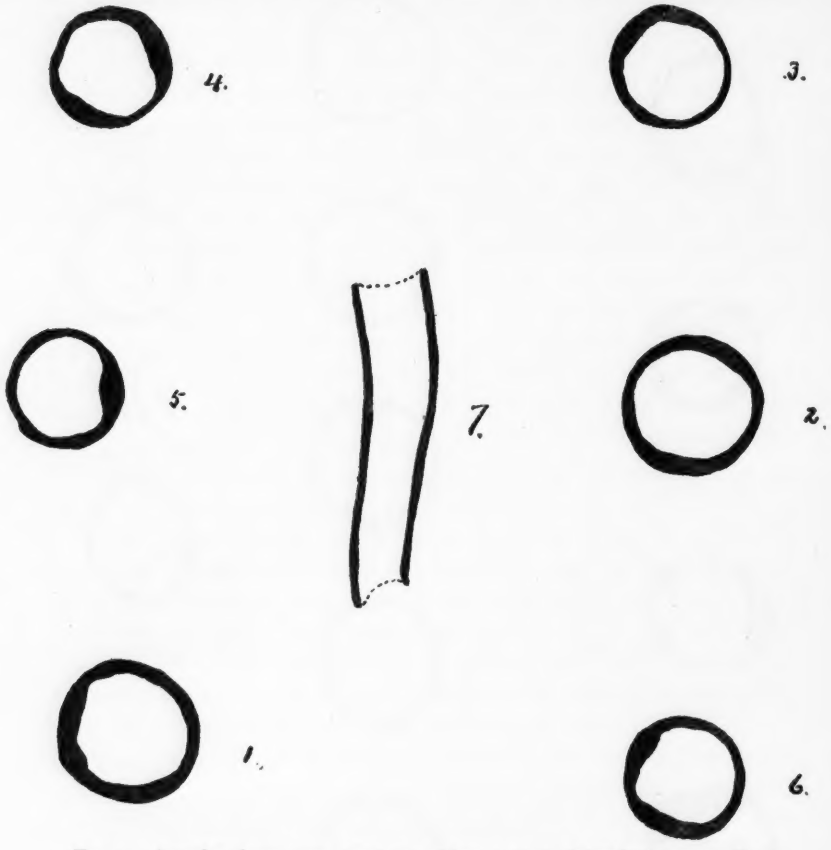

FIG. 3. Arteries from man aged 75. Nos. 1 to 6 abdominal aorta; No. 7, common iliac. Moderate diffuse and nodular endarteritis. Pouching in iliac obliterated by internal pressure.

Pearly thickenings of the intima without atheroma, the true endarteritis chronica nodosa, occupied the whole thickness of the intima and projected to a considerable extent into the lumen of the aorta.

The media was unaltered and formed a circular band of tissue with no evidence of bulging opposite the intimal thickening (Fig. 4).

On the other hand, in the cases of chronic endarteritis in which a degenerative softening had taken place in the deep structures, the media was found to be involved to a considerable degree and showed distinct evidence of thinning. In these specimens the deep intimal degeneration was so closely involved with a degeneration in the inner layers of the media that these two portions were fused, making it appear that the thickening and degenerative process was situated in the intima alone. The media was obviously thinned opposite the degenerative process, but there was nothing in the remaining media to indicate a primary weakening or change in these muscle

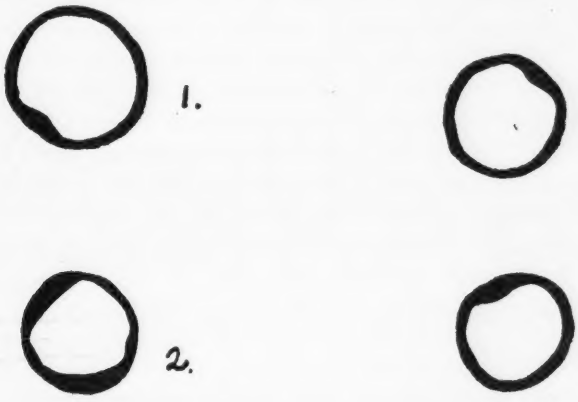

FIG. 4. FIG. 5.

FIG. 4. Female aged 53. No. 1 thoracic aorta. No. 2 abdominal aorta. Showing early endarteritis in the thoracic portion projecting into the lumen, also irregular lumen of extensive sclerosis of abdominal portion.

FIG. 5. Female aged 26. Thoracic aorta. Showing early endarteritis. Endarteritic areas project into lumen of vessel.

fibers which could secondarily induce intimal hyperplasia to excess with degeneration (Figs. 1 and 2).

By these same methods I also examined some vessels, particularly the iliac arteries, in which known lesions of the media were present. Some of these vessels showed typical Moenckeberg's arteriosclerosis with degeneration and calcification of the middle coat, while the

intima showed little or no change over these areas. Macroscopically, and in the relaxed condition, these vessels showed small pouchings and transverse grooves on the inner surface. There were no nodular thickenings of the intima to be seen. When, however, these vessels were subjected to a hydrostatic pressure equal to one hundred and sixty millimeters of mercury and allowed to freeze, the longitudinal section of the artery showed quite a smooth intimal surface. The ridges and pouchings in the vessel wall had been obliterated (Fig. 3). Obviously here the more normal part of the vessel wall had, under pressure, dilated equal to the pouching present in the diseased areas. This is exactly what we should expect in comparing the results with the histological preparations. It has been repeatedly commented upon by various authors that the sclerosed and calcified areas of the media in Moenckeberg's disease diminish the elasticity of the media and fix the muscular tissues in a rigid state. In life this sclerosis takes place while the vessel is under pressure and hence the tissues become fixed and rigid while fully dilated. Under ordinary conditions of life, these sclerosed areas need not be more widely dilated than the adjoining more healthy arterial wall, as is shown in our experiments. Immediately on relaxing the blood pressure at death, the active and more healthy portions of the vessel wall contract, leaving the sclerosed portions as transverse areas to form the pouchings, as is seen at autopsy.

Here, then, we have a process exactly contrary to Thoma's theory. We find that in the areas where the media is diseased there is a pouching of the wall when the vessel is relaxed, and that these areas of medial disease are not covered by a thickened intima of compensatory hyperplasia.

But besides this macroscopic demonstration, we possess clearer evidence that the lesions of the intima are to a great extent independent of medial changes. Dividing the diseases occurring in the intima of arteries into the productive and the degenerative types, we shall, in this paper, confine ourselves to those lesions which lead to the development of new tissues. Nor is it within the province of this discussion to consider the final outcome of intimal hyperplasia, whether this leads to a process of degeneration or continues in the production of new tissue.

One meets not infrequently with specimens of endarteritis chronica nodosa in the aorta in which various grades of the disease can be recognized. The inner surface of the aorta is studded with pearly intimal thickenings of all sizes and in all stages, from the earliest beginning to the large plaques showing deep fatty change.

If, however, we confine our attention to those small islands of fibroses (be these of endothelial or connective tissue origin), which are just visible macroscopically, and study the microscopic characters of these specimens, we find that the diseased process is confined wholly to the intima with no change in the media. It is well to exclude, as far as possible, the presence of senile changes in the media of the aorta, and hence only vessels of adults under thirty-five years should be examined. By none of the known methods of staining can we demonstrate associated lesions in the media in these early processes of endarteritis. Fat and hyaline changes are wanting, calcareous salts have not been deposited, and the elastic fibers of the media show no inequality in staining. In short, we have to deal with a process which is confined to the intima.

This disease of the intima falls into two classes, according to the classification of Jores, (1) the hyperplastic, and (2) the regenerative.

Jores clearly points out that the hyperplastic thickening of the intima, which leads to an excessive development of the musculo-elastic layer, is a "hyper-physiological" process. There are many factors in the life-history of every individual which throw an increased strain upon this muscle layer of the intima of the arteries, and which, if not too persistent, lead to this natural hypertrophy. When, however, these factors are excessive, degenerative changes develop in the hypertrophied tissues. This result is a common, if not the usual finding in hyperplastic intimal thickenings in later life.

The regenerative lesions of the intima are processes taking place in the tissues superficial to the musculo-elastic layer, and occur quite apart from any changes in the deep intimal tissues. This regenerative process is regarded by Jores and others as a connective tissue proliferation of the nature of an inflammation but lacking, at the same time, the features of inflammation, as found in other tissues. A study of the earliest changes occurring in the development of these minute plaques shows that they are a result of the proliferation of

the superficial cells. At the beginning, only a single type of cell, which by some is regarded as of endothelial origin, by others as of connective tissue origin, is found to take part in the proliferative change. Later, fine elastic fibrils are found to permeate the intimal plaque.

Such proliferative changes are most commonly met with in bacterial infections, and occasionally in association with poisons.

These lesions may progress to a considerable size before there is any evidence of change either proliferative or regenerative in the media, and no rule can be followed which will indicate the changes in the media during any particular stage of the disease. Moreover, it is not possible to determine any association between the development of these medial lesions and those of the intima. It would seem that, in these cases, the conditions in the media and in the intima arise wholly apart from each other, though they may result from a common cause.

From these cases we must clearly differentiate the lesions which arise in the media as a result of advanced intimal change. This is familiar to all and is quite a different process. When intimal lesions have been progressive and the thickening of this layer has become so advanced that nutrition is no longer able to pass from the lumen of the vessel to the innermost border of the media, nutritional degeneration, with a deposit of fat in the tissues, will take place. In these instances, the medial lesions are obviously secondary to an intimal disease.

Let us, for a moment, give our attention to the diseases affecting the media of the peripheral vessels and determine the part played by this coat in intimal hyperplasias. For this study the iliac, femoral, mesenteric or brachial arteries and their branches serve very well. Particularly in the case of the iliac and femoral arteries, can the medial diseases be studied from their first beginnings to the severest lesions.

The media of the peripheral vessels, as has repeatedly been indicated, is a functional muscular structure which has to do with the control of the blood supply of the part. Under conditions of stress the muscle tissue of the artery is active, and controls the amount of blood passing to the limbs or organs. Where the blood pressure

and the velocity of the blood is increased, a smaller arterial lumen is required to give an equal amount of blood to the tissues, as under normal conditions. This diminution of the lumen is controlled alone by the media. Under ordinary circumstances the media can adequately control this blood supply, but when from various causes, such as hypertrophy of heart, Bright's disease, or excessive physical work, this blood pressure remains high for a long time, the media reacts by hypertrophy while the nutrition to its walls is adequate. When, however, the nutrition fails, this hypertrophied media slowly undergoes retrogressive changes. In the first degenerative changes of the media, the muscle fibers show an accumulation of fat granules in their protoplasm which continue to fill the cells until the muscle fibers undergo complete destruction. Later these areas of degeneration become calcified and in the superficial vessels are readily recognized as beaded tubes.

Such degenerations of the media are not accompanied by any inflammatory reaction and appear to be the result of over-strain or over-stimulation of the muscle elements. The condition can, in short, be considered as a process of fatigue of the muscle fibers coupled with nutritional disturbance. In consequence of the medial fatigue, there is a localized weakness of the wall, which is evidenced by the pouching that results in the areas of least resistance.

These progressive medial degenerations occurring in the peripheral vessels are readily studied in all the stages, and not infrequently several stages can be observed in the same artery. It is occasionally found that vessels show a considerable microscopic degeneration in the media before any pouching or dilatation results. This is probably due to the fact that the media had previously undergone an hypertrophy sufficient to withstand the heightened blood pressure even after some of the muscle lamellae had been destroyed.

These peripheral vessels showing medial degeneration show irregular conditions of the intima. In many cases the intima is found in a normal condition over lesions of various grades in the media. I have observed the successive changes in the media, from those showing the earliest sign of hypertrophy to the advanced changes in the media with pouching and calcification, without meeting with any condition of endarteritis nodosa or diffusa over the

lesion. It is true that vessels with medial calcification not infrequently show a condition of endarteritis, but this endarteritis is found to bear no direct or secondary relation to the medial disease, save that both may be produced by the same injurious agent.

What is true of medial degeneration and dilatations of the peripheral vessels also holds true of the aorta. In the diffuse though not severe degenerations of the media in the aorta in old age, there is sufficient reason to believe that the arterial wall is materially weakened and that the vessel may be locally or diffusely stretched. Nevertheless, from this medial process alone we cannot predict what changes have occurred in the intima. Occasionally in such vessels the intima is without endarterial change.

Saccular aneurysms of the aorta not uncommonly show some thickening of the intima, but, as has been shown by others (Osler, Chiari), these aneurysms are most frequently the result of a syphilitic mesaortitis, and the intimal overgrowth is associated with the syphilitic process before an actual weakness or stretching of the vessel wall has shown itself. The mesaortitis is essentially an inflammatory process to which the neighboring intima reacts.

From our observations on the medial lesions occurring in the human blood vessels, we are unable to make any general statements associating these with the thickening of the intima, nor does there appear to be any "compensatory" reaction on the part of the intima due to medial weakening.

The experimental lesions in the arteries, too, have been of considerable value in the study of intimal hyperplasias. Some have contended that, as spontaneous disease may occur in the arteries of the experimental animals, or that the lesions experimentally produced were not comparable to the disease in man, no conclusions could be drawn from the work in experimental arteriosclerosis. Without discussing the right of this opposition, we need only point out that the study of the arterial lesions, as they occur in animals, has assisted to a great extent our knowledge concerning progressive and retrogressive changes of the various coats of arteries in general.

Various investigators studied the arterial lesions following mechanical injury (D'Anna, Malkoff, Israel). In all of these, varying grades of inflammatory reaction were obtained. The inflammation

extended from the adventitia through the media to the intima. When healing was allowed to take place, there was a connective tissue replacement of the arterial wall, while the intima showed a nodular thickening over the injury. In some instances (Israel) the intimal thickening was followed by degenerative changes. It appears evident that in the intimal thickening we have to do with an inflammatory repair of the injured coat, although in the later stages all evidence of acute inflammation is wanting. It is probable that all vessels which are disturbed from their normal bed of *vasa vasorum* will later show inflammatory and degenerative processes.

Similar inflammatory changes of inconstant characters were obtained when large nerve trunks (sciatic) were interfered with (Bervoets and Fraenkel). However, it was later shown by Czylharz and Helbing that the vascular reactions were secondary to trophic lesions (ulcers) which occurred on the limbs of the animals. When no trophic changes were present, no arterial lesions developed. Hence the loss of nervous control of the arterial wall with the dilatation of the channels, was in itself not sufficient cause to produce intimal hyperplasia.

The arterial changes produced by the inoculation of adrenalin are interesting and have been thoroughly studied by some fifty-five investigators (Saltykow). The nature of the lesions is well known. In the majority of cases, the thoracic aorta was the site of predilection, though in a number of instances some of the peripheral vessels were also affected. These lesions most frequently consisted of primary degenerative processes in the media, affecting both the muscle and the elastic tissues. The muscular elements were first destroyed, and the whole vessel wall became thinner. In the early stages, with the loss of the muscle elements, the elastic fibers of the media were crowded closer together. Eventually the affected area became calcified and remained as an isolated mass in the middle zone of the media, with relatively healthy adventitia and intima on either side of it.

In only a few instances was any thickening of the intima observed over or associated with the medial degeneration. This thickening of the intima was in part a hypertrophy of the deep muscle coat of this layer along with some fibrous tissue overgrowth close beneath

the endothelial surface. In some instances the hypertrophy of the musculo-elastic layer was quite remarkable, and in studying the various stages of the thickening of the intima in these lesions it was seen that the muscle hypertrophy preceded any development of connective tissue in the intima. Later, this development of muscle tissue in the intima was followed by degenerative changes simulating atheroma.

Although the medial degeneration was the most constant observation made by various investigators, none of them found sufficient evidence in their studies to associate relationship between the medial degeneration and the thickening of the intima. Ziegler, however, noted in his specimens, that the intimal overgrowth assisted, in some measure, the smoothing out of the depressions produced by the giving way of the media.

In other experiments we have more direct evidence of the production of intimal hyperplasias. Sumikawa found, when the femoral artery of rabbits was laid bare and was painted with a four per cent. solution of silver nitrate or with turpentine, that a localized inflammation followed in the tissues with an intimal thickening. The author concluded that the intimal reaction was a secondary one, following the general inflammatory process of the other coats.

Better results were obtained by the bacterial or toxin inoculations. In using various kinds of organisms, *B. typhi, Streptococcus pyogenes, Staphylococcus aureus* and others, it was found that considerable change occurred in the intima of the arteries, while the media showed little or no change (Saltykow, Crocq, Gilbert and Lion, Sumikawa, Klotz). Endothelial proliferations were seen early followed by an edematous or hyaline-like degeneration of the newly developed layer. In some cases a fatty degeneration was noted in the intima, so that the lesion resembled very closely the early atheroma of man. There was apparently a selective action on the part of the bacterial poisons which acted more particularly upon the tissues of the intima. On no account could the intimal hyperplasia, with splitting of the intimal elastic lamina, and its secondary degeneration be associated with a weakening or disease of the media. The hyperplasia of the intima was, in these infections, primary.

In my own experiments, which had been undertaken to eliminate as far as possible the action of poisons and toxins, it was found that increased work on the arterial wall, when carried on for an excessive period, had a double effect on the arterial tissues. On the one hand, it was found that, in those arteries which were subjected to the greatest stress and where that stress no longer acted as an irritant but as a damaging factor, the middle coat of the artery, and particularly the muscle cells therein, showed degenerative changes, even to destruction. The appearance of these vessels was quite similar to that which was obtained when adrenalin and other pressure-producing drugs were inoculated into animals. Quite similar to the adrenalin experiments, there was relatively little reaction in the intima over the medial lesions. On the other hand, in some of the smaller arteries, particularly in the carotid, where the damaging factors were not so severely felt, the artery reacted to some degree by a hypertrophy of its various coats. This hypertrophy was to be seen especially in the musculo-elastic layer of the intima. The size of the muscle bundle was distinctly increased and there appeared to be not only an increase in the size of the muscle cells but also in their number. This, in itself, assisted in thickening the vessel wall. Later changes were, however, prone to occur. In irregular areas this hypertrophied musculo-elastic layer became swollen and with ordinary staining appeared edematous. The tissue was loose and the connective tissue fibres about this layer had a hyaline appearance. In these areas great quantities of fat were readily demonstrated and it was found also that the internal elastic lamina had split into several layers, changes quite similar to those found in this band in arteriosclerosis in man. Other than the hypertrophy, the media showed no definite change, although in the areas where the fatty degeneration of the intima was extensive, a similar condition was found in the inner layer of the media bordering the intima. The mid and external zones of the media showed in no instance any feature which would indicate a weakening, and the slight amount of fatty change seen in the inner zone of the media was readily recognized as being secondary to the lesion in the intima (Plate LXIX, Figs. 1, 2 and 3).

Some have referred to the apparent intimal hyperplasia which

occurred in transplanted vein segments as instances of Thoma's compensatory intimal hyperplasia. Since the valuable work of Carrel and Guthrie, indicating the manner in which arteries and veins might be successfully sutured, a number of operators have devised unique experiments on tissue transplantation. Fischer and von Schmieden have recently repeated the experiments of arteriovenous anastomosis and of the transplantation of a vein segment into the course of an artery. After allowing a complete healing of the sutured edges, the transplanted veins were examined after varying periods of time. The experiments were undertaken in answer to two questions: (1) Does a healthy vein become unduly dilated (varicosed) when under high pressure? and (2), Does the intima of the vein show a functional connective tissue hyperplasia? From their observation in their own experiments, they conclude that veins when under arterial pressure do not become unduly dilated, but after a time show a narrowing of the lumen, due to hypertrophy of the media by an increase in the muscle and connective tissue. The adventitia too was found to increase its connective tissue elements. The authors could find no compensatory hyperplasia of the intima, although nodular thickenings with inflammatory surroundings were occasionally observed. These nodules of the intima occurred most frequently about the sutures, and not in the most distended part of the vein, which was away from the suture line. In other words, the reaction in the vessel wall due to the increased function was found mainly in the muscle cells as an hypertrophy.

With reference to Thoma's views upon compensatory hypertrophy, it may be said that Adami has expressed views which, while subscribing to the idea that compensatory hypertrophy occurs, tend to ascribe it to other reasons. Both of the theories of Thoma, and those of Adami, assume a medial weakening antedating the growth of the tissue cells of the intima. Thoma contends that this weakening led to a dilatation of the artery with sufficient slowing of the blood current to cause reactionary proliferation of the intima; Adami, on the other hand, believed that the medial weakening and dilatation caused a tugging or stretching of the intima and its cells, and that these physical agencies were the stimuli for growth. However, the fundamental principle upon which both these theories are

based, namely, a primary weakening of the media, remains unproved, and I would even say stands contrary to many observations on this point.

No one will doubt, I believe, that active work associated with an adequate nutrition will bring about hypertrophy or hyperplasia of many tissues. This increase of work may be, on the one hand, of a physical nature, or, on the other, of increased secreting function. In each case, the result is the same. While the nutrition remains sufficient for the needs of the tissue and the hypertrophied structures, a progressive increase in the number and size of the cells results. In other words, work in its various forms acts as a stimulus for cell growth, as long as the nutrition is adequate.

The tissues most affected in the arteries by the stress of physical agents, such as high blood pressure, are the muscle cells and, to a less extent, the elastic tissue fibers. When arteries are stimulated in this manner, these cells are the first to react, as is evidenced by the early hypertrophy of the media and of the musculo-elastic layer. These same cells are also those which suffer the effects of overstimulation, or fatigue, and in them degenerations appear early. In examining my specimens, Professor Adami, while recognizing the hypertrophy of the musculo-elastic layer, believed, nevertheless, that the overgrowth of the overlying connective tissue of the intima proper, as it showed no earmarks of inflammation, indicated a strain hypertrophy.

Much of this newly developed muscular tissue in the deep layer of the intima is followed later by very extensive fatty degeneration, so that much of this tissue is again lost. Over the hypertrophied muscle layer there is at times a development of a loose fibrous stroma, appearing hyaline or edematous. There is every evidence to believe that this superficial tissue, be it of endothelial or connective tissue origin, has developed secondary to the degenerative processes in the deep part of the intima.

Notwithstanding this, Adami believes that a share of the increased work of the arteries falls upon the superficial cells of the intima, and that the stretching of this lining causes a proliferation of the cells.

Concerning the reaction seen in the intima in syphilitic disease

of the aorta, we incline to the view that it is secondary to the inflammatory processes of the media. Our increasing familiarity with syphilitic mesaortitis indicates that the lesions in the arteries are of an infective nature and that this infection lies in the adventitia and media. With this infective process there is truly a destructive process in the media, but there is also a concurrent inflammation which we feel convinced affects the intima.

The effect of moderately increased work upon the arterial walls leads to an hypertrophy of the muscle elements, giving way not infrequently to a later degeneration. The degeneration becomes most evident in the musculo-elastic layer which gives rise to atheroma. The growth of connective tissue elements over this degeneration is secondary.

When increased work exceeds the capabilities of the arterial tissues, degenerative changes set in from the first and no intimal hyperplasia is evident.

CONCLUSIONS.

We agree with Jores and others that not one but many factors may be at work leading to intimal hyperplasia. Among these factors may be mentioned infection, bacterial toxins, organic poisons, inflammation and increased arterial tension.

The theory of Thoma that the connective tissue developed in the intima is compensatory cannot be sustained.

From the evidence which we have at hand it is not possible to state that the proliferative changes in the intima are uniformly secondary to the weakening of the media.

Common influences may act simultaneously upon the media and the intima.

Progressive medial degeneration of the peripheral arteries (Moenckeberg's sclerosis) is the result of muscle fatigue coupled with nutritional disturbance.

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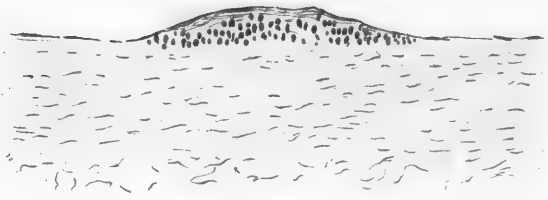

FIG. 1.

FIG. 2.

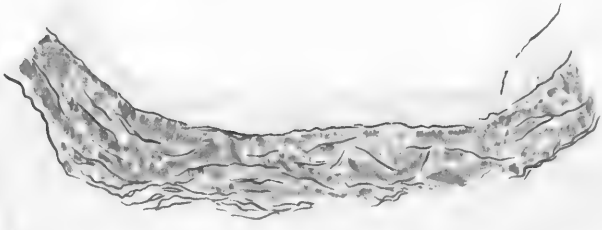

FIG. 3.

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EXPLANATION OF PLATE LXIX.

The tracings, including those in the text figures, are from impressions made from transverse sections of different aortas while frozen under pressure.

FIG. 1. Carotid artery of rabbit, daily inverted for five minutes for 120 days. Showing hypertrophy of musculo-elastic layer.

FIG. 2. Carotid artery of same animal as Fig. 6, in which hypertrophied musculo-elastic layer shows much fatty change.

FIG. 3. Carotid artery of rabbit treated for 135 days, in which the thickened musculo-elastic layer is very fatty, with a thickening of the subendothelial layer (endarteritis chronica).

OBSERVATIONS UPON THE NOGUCHI MODIFICATION OF THE WASSERMANN COMPLEMENT FIXATION TEST IN THE DIAGNOSIS OF LUES IN THE MILITARY SERVICE.[1]

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In November, 1909, the Noguchi modification of the Wassermann complement fixation test for the diagnosis of lues was adopted as a routine measure in the Laboratory of the Office of the Surgeon General of the Army, and it is the purpose of the writer to give in detail the results obtained to date with this test, together with some general deductions concerning the use of the test in the diagnosis and clinical study of the disease. The Noguchi modification was adopted after a careful consideration of the original Wassermann test and other modifications, and our experience with it has been most favorable. The results that we have obtained have been such that we are forced to believe that most of the adverse criticism of the method has resulted from its improper use in the hands of those who have not been carefully trained in its application, and it will be evident from our results that this method of making the complement fixation test is of the greatest value in routine diagnostic work in the military service.

Technique Employed.—The technique we have employed in making the test is largely that recommended by Noguchi, although half of our tests have been made with liquid amboceptor, and all of them with liquid antigen. However, the writer has used antigen dried upon filter paper in a large number of tests with perfectly satisfactory results and has found that amboceptor dried upon filter paper gives as good results as does the serum, while it is much more stable.

[1] Published with permission of the Surgeon General, U. S. Army. Received for publication July 15, 1910.

The *antigen* used has been an extract of luetic fetal liver in absolute alcohol. This antigen has proven most satisfactory, but in using it we have always inactivated the serum to be tested, for in a few parallel cases tested with active and inactive serum, we found that erroneous results were obtained a few times with the active serum. As a matter of fact, receiving our specimens, as we do, from army posts scattered all over the United States, most of the serum reaching us is already inactivated.

The *complement* used has been guinea-pig serum, while the *amboceptor* was prepared by immunizing rabbits to human red blood corpuscles. We have found that four intraperitoneal injections of five cubic centimeters of washed human red corpuscles will give a hemolytic serum of such strength that when impregnated into filter paper, a piece five millimeters square will produce hemolysis of one cubic centimeter of a one per cent. suspension of human red corpuscles. The animals differ somewhat in the strength of the serum which they furnish, but if the sera of three or four animals be mixed, the resulting mixture will be found of suitable strength.

The *blood suspension* is prepared by adding one drop of blood to each four cubic centimeters of salt solution, and the writer has found that a suspension prepared as recommended by Noguchi is always satisfactory.

The method of making the test and the principles involved will not be considered at this time, as they are familiar to all who are interested in the Wassermann reaction.

The writer has become impressed during his work with the *quantitative* nature of this test, and in every instance in which anomalous results have been obtained, and such results have but rarely appeared, it has been found that the fault lay in some quantitative error made by himself, and not inherent in the method. A careful repetition of the test has always resulted in clearing up the difficulty and in demonstrating an error in the technique. While this test is much more simple and practical than the original Wassermann test, it requires the utmost care in its application, and it is the writer's belief that it should be done only by trained men in a properly equipped laboratory. Phenomena are not infrequently observed which can be interpreted only by one who is trained in serum work,

and a whole series of tests may be rendered valueless by a slight loss of strength in the complement or by a deterioration of the antigen or amboceptor. All of the elements used in the test have to be titrated frequently, and such titration is possible only when the observer is trained in this line of work.

The tests made in this laboratory may be divided into the following classes: Group I. Cases in which the clinical diagnosis was lues or suspected lues, or in which there was a history of previous infection (this includes latent cases). Group II. Cases in which the clinical symptoms or diagnosis were of some other disease. Group III. Cases known not to be luetic in nature.

The total number of individuals examined to September 12, 1910, has been 639, of which 319 were positive, and 320 were negative. It may here be stated that *no case has been termed positive unless there was absolute inhibition of hemolysis. Weak and partial reactions have occurred, but they have been reported only where the effect of treatment was requested, never where a diagnosis of lues was in question.* By insisting upon absolute inhibition of hemolysis as indicating a positive reaction, we have undoubtedly missed some cases of lues, but we have not felt justified in basing a positive report upon anything less.

Group I. Cases in which the diagnosis was lues or suspected lues, etc.—Of this group of cases, 391 were examined, of which 316, or 80 per cent., gave positive results. The cases were divided as regards the stage of the disease as follows:

Stage of disease.	No. of cases.	Positive.	Negative.	Per cent. positive.
Primary	90	65	25	72.
Secondary	163	143	20	87.7
Tertiary	74	61	13	82.
Latent	55	39	16	72.
Congenital	9	8	1	88.8
Totals	391	316	75	80

Group II. Cases in which the diagnosis or clinical symptoms were those of diseases other than lues.—Tests were made upon 178 individuals belonging to this group, with a positive result in three

cases. Of the three cases, two were suffering from malarial fever of tertian type, and the tests were made during the febrile stage; and one, from frambesia. Both malarial patients became negative when the temperature reached normal. Very weak reactions have been observed in a few other cases, but they were so weak that they could never have caused any difficulty in interpreting the result, and as such reactions would never lead to a positive report, we do not consider them of any clinical importance.

Group III. Cases known not to be luetic in nature.—Tests have been made (for experimental purposes) upon seventy healthy individuals in whom lues could be positively excluded, and a negative result was obtained in every instance.

Analysis of the Results.—It will be of interest to consider our results a little more in detail and to compare them with those obtained by other investigators. In doing this we will first consider the results obtained in Group I, and later the results in the other groups mentioned.

Group I. Primary Stage.—We have made no attempt to separate our cases into groups in this stage, as has been done by Pedersen, but we have considered all cases as primary which have been tested before the development of secondary symptoms. Of our cases, ninety were tested during the primary stage of lues, of which sixty-five, or 72 per cent., gave a positive reaction. However, these figures include several cases tested within a few days after the appearance of the initial lesion, too early for the reaction to appear, and several in which vigorous treatment had been pursued for some time, so that subtracting from the total number of primary cases the fourteen of this character, we obtained sixty-five positive reactions in seventy-six cases, or 85.5 per cent. Table I gives the results obtained by several other observers in the primary stage of the disease for purposes of comparison.

From the above table it is evident that the percentage of positive results in primary lues varies from 38.6 per cent. to 100 per cent., in the hands of various observers, while of the total number, 812, there were 575 positive reactions, or 70.8 per cent. In most of the tests given in the table the technique employed was that of the original Wassermann test, and the differences between the results thus

TABLE I.

Results of Complement Fixation Test in Primary Lues.

Name of observer.	No. of cases.	Positive.	Per cent. positive.
Arning	48	25	60
Bering	56	47	84
Boardman	13	6	46
Boas	50	30	60
Bruck-Stern	27	13	48.5
Detre-Brezowski	43	21	49
Fischer-Meier	8	6	75
Fox	7	7	100
Grosser	20	19	95
Hancken	17	15	88
Hoehne	44	17	38.6
Hoffman-Blumenthal	12	6	50
Jesionek-Meirowsky	30	20	66.6
Kaplan	138	125	90
Lesser	56	39	69
Marcus	35	22	63
Meier	25	17	68
Merz	64	48	71.6
Müller	14	5	45.5
Noguchi	70	65	92.8
Schonnefeld	19	9	47
Swift	16	13	81
Totals	812	575	70.8

obtained and those in which the Noguchi technique is used will be considered later in this contribution, but it may be stated here that the writer believes that the results are practically identical with both methods, with the advantage slightly in favor of the Noguchi system.

The results obtained in this laboratory are in agreement with those obtained elsewhere, sixty-five of our cases, or 72 per cent., in the primary stage giving a positive result. It is undoubtedly true that the percentage of positive results varies more in the primary stage of lues than in any other, and for this there are many reasons, irrespective of the method employed. Many of the negative results are due to the fact that the test is made at too early a period, while in some cases, early treatment, if vigorous, will obscure the reaction. In untreated cases the writer has twice observed a negative result followed by a strong positive result within two weeks, and several in which a negative reaction was changed to a positive after the development of secondary symptoms.

Time of Appearance of the Reaction.—We may here consider

our results as regards the time of appearance of the reaction in the primary cases. In most of our cases the infection had existed for some time before the test was made, for which reason it is impossible to state definitely just when the reaction became positive, but in thirty-one of our cases which were positive, the test was made within thirty days after the appearance of the initial lesion. The following table (Table II) gives our results in these cases.

TABLE II.

Date of Appearance of Positive Reaction in Thirty-one Cases of Lues.

Days after initial lesion.	No. of cases positive.	Days after initial lesion.	No. of cases positive.
5	1	20	1
8	2	21	2
11	2	22	1
13	3	23	1
14	1	25	2
17	2	28	1
18	2	29	2
19	2	30	6

It should be remembered that the reaction may have been present in any of the cases before we tested the blood, the table giving the first examination made in each case. From the table it will be observed that the earliest period in which a positive reaction was obtained was five days after the initial lesion was first noticed by the patient, and it should not be forgotten in considering these figures that our index of a positive reaction was absolute inhibition of hemolysis. Weak and partial reactions were observed in several instances within from seven to fifteen days after the appearance of the chancre, but these have not been considered in the tabulation of our cases. Lesser and Levaditi both report a positive reaction upon the eighth day after the initial lesion appeared, but so far as the writer knows, our case, in which a positive result was obtained upon the fifth day after the chancre appeared, is the earliest upon record. In Boardman's cases the reaction generally appeared within two or three weeks after the chancre, and this has also been our experience.

If, as appears to be proven by Neisser's work with apes, the positive reaction means a general intoxication with the luetic virus, it is

easy to understand why the reaction appears much earlier in some patients than in others, and also why it disappears spontaneously in many instances. In other words, the virulence of the virus and the reaction of the system to it will have much to do with the appearance and disappearance of the reaction. While we have secured a positive reaction as early as five days after the appearance of the initial lesion, we have observed a negative result in luetic patients, who have never taken specific treatment, in whom the infection had existed for as long as eight years. Table III gives our results in seventy-one cases of lues in which the test was made thirty-one days or longer after the appearance of the chancre. None of these patients had been well treated with mercury.

TABLE III.

Result of Complement Fixation Test at Various Periods after Initial Lesion.

Days after initial lesion.	Positive.	Negative.	Days after initial lesion.	Positive.	Negative.
32	1		150	1	
33	1		180	4	
34		1	210	1	
35	2		270	2	
38	1		300	1	
42	1		330	1	
46	2		365	5	
49	1		380	1	
50	1		547	1	
55	1		730	3	
60	2	1	912	1	
70	2		1,095	1	
72	1		1,460	2	
87	1		1,825	2	
90	7	1	2,190	2	
99	1		2,555	1	1
100	1		2,920	1	1
110	1		3,653	1	
120	4		4,015	1	
144	1				

It is interesting to observe that of seventy-one patients in whom a clear history of lues could be obtained, and who were tested at a period later than one month after the appearance of the initial lesion, only five gave a negative reaction, while of thirty-six patients tested within one month after the appearance of the chancre, the same number, five, gave a negative reaction. In short, positive reactions

are more numerous the longer the infection has lasted, and this agrees with the results of every observer, without reference to the method employed in making the complement fixation test.

Group I. Secondary Stage.—We have tested 163 patients in the secondary stage of lues with a positive result in 143 cases, or 87.7 per cent. If we eliminate from this class the patients who had received or were receiving specific treatment, sixteen in number, we have remaining a total of 147 cases with 143 positive results, or 97.2 per cent.

The results of several other investigators who have examined luetic patients in the secondary stage of the disease are given in Table IV for purposes of comparison.

TABLE IV.

Results of Complement Fixation Test in Secondary Stage of Lues.

Observer.	No. of cases examined.	Positive.	Per cent. positive.
Arning	107	99	93
Bering	113	111	98
Boardman	26	20	77
Bruck-Stern	115	101	87.1
Blumenthal-Roscher	131	130	99
Butler	25	24	96
Fox	37	36	97
Grosser	36	33	92
Hoene	376	260	79.1
Hoffman-Blumenthal	99	82	82
Jesionek-Meirowsky	93	90	96.7
Kaplan	281	242	86
Lesser	204	186	91
Ledermann	110	108	98
Marcus	14	10	71
Meier	84	79	93
Merz	377	366	97
Noguchi	197	190	96
Schonnefeld	112	112	100
Swift	76	70	92
Totals	2,613	2,349	89.8

From the table it will be seen that the percentage of positive results in the secondary stage of lues varies, according to different observers, from 71 per cent. to 100 per cent., the latter figure being recorded by only one observer. In the tests tabulated, the original Wassermann technique was used, with the exception of Noguchi's

tests, and it will be observed that the results secured by the latter and our own are practically the same as those of the majority of other observers. It is also evident that the secondary stage of the disease gives a positive result in a very large proportion of cases, but it must be admitted that even here a negative result is occasionally encountered.

Group I. Tertiary Stage.—We have tested seventy-four individuals in the tertiary stage of lues with a positive result in sixty-one, or 82 per cent. Some of these patients had had more or less specific treatment, and some of them were undergoing treatment at the time the test was made, but if we should eliminate these from the total number, so few would be left that the figures would have little, if any, significance. It should be understood, therefore, that the figures as they stand represent both treated and untreated cases.

Table V illustrates the results of other observers in this stage of the disease.

TABLE V.

Results of the Complement Fixation Test in Tertiary Lues.

Observer.	No. of cases examined.	Positive.	Per cent. positive.
Arning	30	27	90
Bering	45	37	82
Boardman	32	30	94
Bruck-Stern	47	27	57.4
Blumenthal-Roscher	9	9	100
Butler	17	16	94
Fleishman	41	40	98
Fox	32	23	71
Grosser	19	11	57.8
Hoehne	33	21	63.6
Hauck	13	10	77
Heller	18	14	72.8
Hoffmann-Blumenthal	26	23	88
Jesionek-Meirowsky	48	42	87.5
Kaplan	191	140	73
Ledermann	78	75	96
Lesser	131	119	90
Ludke	14	11	79
Merz	158	127	80.3
Noguchi	177	159	89.9
Swift	45	37	80
Thomsen-Boas	63	61	96
Totals	1,267	1,059	83.5

From the table it is evident that the percentage of positive results in tertiary lues varies from 57.4 per cent. to 100 per cent., according

to various observers. Our own results agree with those obtained by the majority of the investigators mentioned in the table. With the exception of Noguchi, all of these used the Wassermann technique.

The great variation in the results, obtained by some of the observers mentioned, may probably be explained by their including under the heading of tertiary lues, (1) cases without symptoms, those which had received or were receiving treatment, and (2) cases in which no distinct tertiary lesions had ever appeared. Until our statistics are based upon exactly similar cases we must expect great variation in the results of the test as reported by different observers. Thus Lesser found that of 131 patients in whom symptoms of this stage were present, no less than 90 per cent. gave a positive reaction, while of 425 patients in whom no symptoms were present, only 46 per cent. were positive. In our cases, tertiary symptoms were present in nearly two-thirds.

Group I. Latent Lues.—Under this heading we have placed all cases in which no symptoms were present at the time of the examination, irrespective of treatment, in which a clear history of lues could be obtained. Of the fifty-five individuals in this class, twenty-nine, or 72 per cent., gave a positive reaction. Table VI

TABLE VI.

Results of Complement Fixation Test in Latent Lues.

Observer.	No. of cases examined.	Positive.	Per cent. positive.
Bering	147	75	48
Boardman	18	9	50
Butler	15	8	53
Grosser	35	12	33.3
Gross-Volck	35	6	17
Hancken	20	13	65
Fox	54	25	46
Hauck	31	23	74
Hoffmann Blumenthal	23	12	52
Kaplan	79	41	51
Ledermann	78	36	46
Marcus	23	3	13
Müller	81	29	35.8
Noguchi	265	206	77.7
Swift	39	25	64
Totals	943	523	55.4

gives the results obtained by others in the same class of cases. No attempt was made to divide these cases into early and late latent cases.

With the exception of Noguchi's cases, the technique employed in most of those tabulated was that recommended by Wassermann. It will be observed that there is a great variation in the percentage of positive results obtained by these observers, as would be expected when one considers that in some of the cases there was only a clinical history to go upon in making a diagnosis, and the probability that in many of the cases examined the infection had spontaneously disappeared or specific treatment had been given. Comparing our results with those obtained by the majority of observers with the original Wassermann test, it will be observed that they are somewhat higher, thus proving the greater delicacy of the Noguchi modification in this class of cases.

Group I. Congenital Lues.—Our tests in this group of cases were few in number but the results obtained with the Noguchi modification agree with those of other observers who have used the Wassermann technique. We tested nine cases with a positive result in eight, or 88.8 per cent. Table VII gives the results of other observers in congenital lues.

With the exception of Noguchi's cases, all of the tabulated cases were tested with the Wassermann technique. Comparing our results with these it will be observed that they were practically the same, *i. e.*, eight positive reactions in nine cases, or 88.8 per cent.

The Specificity of the Test.—In considering this portion of our subject we shall first analyze the results of the test in Groups II

TABLE VII.

Results of the Complement Fixation Test in Congenital Lues.

Observer.	No. of cases examined.	Positive.	Per cent. positive.
Arning	5	3	60
Fox	1	1	100
Fischer-Meier	4	3	75
Jesionek-Meirowsky	18	16	88.8
Kaplan	20	18	90
Noguchi	17	17	100
Schonnefeld	3	3	100
Totals	68	61	89.7

and III. In Group II, which includes all cases in which the clinical diagnosis was other than lues, or in which the symptoms were of some other disease, we examined 178 cases, with a positive result in three. Two of the positive results were obtained in malarial fever of tertian type, during the febrile paroxysm, the reaction becoming negative when the temperature reached normal. The other positive result was obtained in a case of frambesia, but the reaction was weak and would not, under ordinary conditions, have been reported as positive. As is well known, the test as performed according to Wassermann's recommendations has proven positive in certain cases both of malaria and frambesia, so that these results cannot be considered as the fault of the Noguchi modification. In eight cases of pellagra the test was absolutely negative.

In Group III, or healthy individuals in whom lues could be absolutely excluded, we tested seventy individuals, with a negative result in every instance. The test was made to determine whether the Noguchi modification ever gave a positive result in such individuals and also to ascertain the keeping power of normal serum. As the question of the specificity of the Wassermann reaction is of the greatest practical importance it will be of interest to give here the results obtained in this direction by the most prominent workers upon the subject. Table VIII gives the result of the test in diseases other than lues.

The technique used in the tests tabulated in Table VIII was that recommended by Wassermann, and we believe that the results we have obtained in this laboratory, *i. e.*, only three positive results in 178 individuals examined, demonstrates that the Noguchi modification of the Wassermann test does not give positive results in negative cases save in rare instances, and then only in the same class of cases as those in which the original Wassermann has given positive results. In leprosy, malaria, scarlet fever, and frambesia, both tests have given positive results in a small proportion of the cases examined, but such results do not injure the great practical value of the test in the diagnosis of lues. A few other conditions have been observed where isolated cases were positive, but we should remember that it is practically impossible to be sure that any of these patients were free from luetic infection and, therefore, it is

TABLE VIII.

The Complement Fixation Test in Diseases other than Lues.

Observer.	No. of cases tested.	Positive.	Negative.	Remarks.
Arning	85	0	85	
Bauer-Meier	350	4	346	1 sepsis. 2 tuberculosis
Bayot-Renaux	220	1	219	Carcinoma
Boardman	49	0	49	
Boas	485	1	484	Scarlet fever
Bruns-Halberstaedter	31	0	31	
Citron-Blaschko	156	0	156	
Fox	23	2	21	2 leprosy, weak
Gozony	33	0	33	
Gross-Volk	63	2	61	1 diphtheria, 1 scarlet fever, both dying
Hoehne	320	3	317	1 scarlet fever, 1 papule, penis: 1 ulcer
Heller	10	0	10	
Hoffman-Blumenthal	35	2	33	2 frambesia
Ledermann	250	2	248	1 leprosy, 1 lues?
Merz	347	0	347	
Schonnefeld	41	0	41	
Swift	85	0	85	
Torday	100	0	100	
Wassermann	1,010	0	1,010	
Totals	3,693	17	3,676	

hardly fair to urge such instances as weakening the value of the test. We believe that it must be evident to any fair observer, from the statistics here given, that the Wassermann test is of the greatest value in the diagnosis of lues and that the results secured with the Noguchi modification are practically the same as those with the Wassermann technique.

The diseases of non-luetic nature in which a positive reaction has been most often obtained are scarlet fever and leprosy, although Bohm claims to have obtained sixteen positive reactions in forty-six patients suffering from malaria, or 35.5 per cent. As regards leprosy, it has been observed that the reaction occurs more frequently in the tubercular form than in the anesthetic or mixed, and in this form of the disease a considerable number of positive reactions have been reported. According to Maslakowetz and Liebmann, the percentage of positive results in leprosy varies with the antigen used, as they found that of sixteen cases tested with alcoholic extract of guinea-pig heart, nine gave a positive reaction; of thirteen cases tested with alcoholic extract of human heart, seven

gave a positive reaction; while of eighteen cases tested with a watery extract of luetic liver, only one gave a positive reaction.

A certain proportion of sera from scarlet fever patients gives a positive reaction with the Wasserman test and the Noguchi modification, the percentage varying with different observers, but here again it is probable that the character of the antigen used has much to do with the number of positive results. Thus Hecht, Lateiner, and Wilenko tested 105 cases, using an alcoholic extract of guinea-pig heart, and obtained but one positive result; while Fua and Koch, using an alcoholic extract of luetic liver obtained forty-five positive results in 353 cases, or 12 per cent.

Much more work needs to be done upon the nature of the antigen before we shall be in a position to explain the positive results obtained in diseases other than lues but we believe that further research will undoubtedly result in knowledge which will enable us to avoid a positive result in all such instances. However, the comparatively insignificant number of positive reactions obtained in a few easily recognized diseases does not render the test of any less value as a practical diagnostic measure, and it is more than probable that many of these positive results could have been avoided by more careful attention to the technique of the test. A large percentage of positive results in non-luetic cases is certain proof of lack of knowledge or care in the application of the test, whether the original Wassermann technique be employed or the Noguchi modification.

The Test as a Control of Treatment.—Our results have been of interest in showing the effect of treatment upon the reaction and we have become convinced that in this test we have a very valuable index of the efficacy of specific treatment in this disease. In Table IX are given our results in cases which were tested after specific treatment had been given, the table showing the length of time the patient had been taking treatment and the result of the test.

This table is of interest both in showing how quickly the reaction may disappear after specific treatment is instituted, and also how long it may persist despite such treatment. It will be observed that negative results were obtained in as short a period as two weeks after beginning treatment, and that similar results were obtained in patients treated for from one to four months, while no less than

TABLE IX.

The Result of Specific Treatment upon the Complement Fixation Test.

Time treated.	No. of cases.	Positive.	Negative.	Time treated.	No. of cases.	Positive.	Negative
2 weeks	2	1	1	40 weeks	1	1	0
4 "	1	0	1	1 year	4	1	3
6 "	2	1	1	14 months	3	1	2
8 "	3	2	1	15 "	1	1	0
10 "	1	1	0	18 "	2	2	0
12 "	4	3	1	2 years	6	5	1
16 "	1	1	0	30 months	1	1	0
24 "	4	3	1	3 years	2	0	2
28 "	3	1	2	8 "	1	1	0
32 "	4	1	3	12 "	1	1	0
36 "	5	4	1				

Total cases with treatment, 52. Positive, 32. Negative, 20. Per cent., 61.

twelve of our negative results in this class of cases were obtained in patients who had been under treatment for less than one year. This fact demonstrates that a negative reaction in such cases cannot be considered as an indication that the infection is cured, for if treatment be discontinued the reaction reappears. It can, however, be used as an index of the efficiency of the treatment employed. The table also demonstrates that irregular and careless treatment has but little effect upon the reaction. In one case, the injection treatment had been used at intervals for two years but the blood serum gave a strong positive reaction. In two other cases, irregular treatment by the mouth had been pursued for eight and twelve years respectively, but both gave positive reactions. On the other hand, one patient who had been carefully treated for one year, gave a negative reaction, and was still negative six months after treatment had been stopped. Negative reactions were also given by a patient treated for two years, and by another who had received careful and continuous treatment for three years. We believe that a positive result invariably indicates the presence of *active* disease, whether manifested by clinical symptoms or not, and that treatment should be continued until the reaction becomes permanently negative. The time required to accomplish this result is very variable, as one patient may have a positive reaction turned into a negative one after only a year's treatment, while others will still show positive reactions after three years or more of careful treatment.

A single negative result is of no value as an index of cure, but the test should be repeated several times at intervals of two to three months, the patient in the meantime having ceased treatment. A negative result in the early period of the disease is of no importance as to cure but becomes more and more so, the later the stage of the disease, the longer the duration of treatment, and when the findings are repeatedly negative. It is evident that one cannot pursue a routine practice as regards the dosage or length of treatment, and that at the present time the only method of actually determining the efficacy of any special mode of treatment is the application of the complement fixation test. It has been shown by Kirolyfi that the amount of mercury present in the blood of a patient taking specific treatment has no effect upon hemolysis, so that a negative result following a positive demonstrates that this drug has a specific effect upon the luetic bodies. It would also appear to prove that it is not necessary for a patient to stop treatment in order to ascertain the status of his serum as regards the complement fixation test.

In those infections in which no symptoms are present, the so-called "latent cases," a positive reaction should always be followed by specific treatment and such treatment continued until the reaction remains negative after repeated examinations. It is true, however, that some patients never give a negative reaction, no matter how long they may have been treated, but such instances are rare, and do not interfere with the great usefulness of the test as a guide to treatment, and we believe that it should be utilized as a routine method of determining the efficacy of treatment in this disease.

It should be remembered that in the early stage of the disease the reaction, if negative after a short course of treatment, generally becomes positive if the treatment is stopped, and, for this reason, is not an indication for the cessation of treatment.

The Interpretation of a Positive Reaction.—We believe that a positive reaction should be considered as one of the symptoms of lues, but a positive reaction alone is not conclusive evidence of the presence of this disease, neither is a negative reaction conclusive as to the absence of the disease. In view of the occurrence of the reaction in such conditions as leprosy, scarlet fever, frambesia, etc., it is evident that we cannot consider it as absolutely diagnostic of

lues, but the presence of the reaction, with or without symptoms, in the absence of any other disease in which a positive result has been obtained, is sufficient to enable us to state that lues is present. In countries in which leprosy and frambesia are prevalent, a positive result should be accepted as indicating lues only when it is possible to exclude these diseases, unless characteristic luetic symptoms are present.

In those cases in which, after the appearance of a suspicious initial lesion, a negative reaction becomes positive, a diagnosis of lues can be made without hesitation, and it is in just this class of cases that the test will prove of great value, in that it will enable us to begin specific treatment before the appearance of secondary symptoms.

The test is of great value in helping us to reach a conclusion regarding those cases in which atypical symptoms are present, and in those giving a history of infection but in which the disease is latent, no symptoms being present. A positive result in the latter class of cases indicates that treatment is needed, while if it is repeatedly negative, it indicates that the infection has disappeared or is cured. Here again it is necessary to exclude such conditions as leprosy, frambesia and scarlet fever.

While some authorities, as Bering, regard the test as absolutely specific, we believe that it is yet too early to make so definite a statement, but we do not believe that the fact that a positive reaction is sometimes obtained in such diseases as leprosy, scarlet fever and frambesia lessens its value from a practical standpoint, as these diseases can be easily excluded, and in scarlet fever the reaction is not permanent. The writer cannot help but believe that many of the positive results reported in conditions other than lues have been due to giving weak and partial reactions an exaggerated value, and it is believed that if absolute inhibition of hemolysis be insisted upon before considering a test as positive, it will be found that the reaction is practically specific.

CONCLUSIONS.

In the vast majority of cases examined in this laboratory the result of the Wassermann test was simply confirmatory of the clinical

findings, but there were numerous suspicious cases, or cases in which symptoms were present and the patient denied a specific history, in which the test was of the greatest value, and it is in such instances, perhaps, that it may be regarded as "the court of last resort" in arriving at a conclusion regarding the nature of the condition present. Certainly if there is any value in experience and statistics, we are justified in regarding this test as the most valuable means we possess of diagnosing lues, and our experience with the Noguchi modification of the test has justified all that has been claimed for that method.

We believe that our results have proven that by the use of the complement fixation test in the military service it is possible to prevent the enlistment of men suffering from latent lues who would otherwise be enlisted; to control specific treatment by using it as an index of the efficiency of such treatment; to clear up the diagnosis of obscure or suspicious cases; and to enable the surgeon to avoid mistakes in discharges for disability in cases suspected of this disease.

Owing to the facility with which clinical observations can be made in armies, and the control that is possible of tested individuals, it is to be hoped that the Noguchi modification of the complement fixation test will be more widely used than it has been in the military services, for which we believe it is especially adapted. So far as we are aware the Medical Department of our army is the first to adopt this test as a routine diagnostic procedure and to apply it in the case of applicants for enlistment.

In conclusion, the writer desires to express his thanks to Dr. Noguchi for the instruction and advice so kindly given during several weeks spent in his laboratory at the Rockefeller Institute for Medical Research.

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THE DISTRIBUTION OF FAT IN THE LIVER.[1]

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We have endeavored in the work upon which this paper is based to obtain some information relative to the fat which appears in the liver at death, as well as its nature and distribution. Fat which can be stained and recognized as such appears in a large percentage of all cases irrespective of age; in the one hundred cases comprised in this series, ninety-eight showed a recognizable amount of it; of twenty successive cases taken from our autopsy series, all showed it. In one hundred cases, fourteen times fat was present in extreme amount, a condition certainly pathological, for in thirteen of these where mention was made of the point, the macroscopic appearance was abnormal; thirty-two times it was considerable in quantity, which condition is probably pathological, for it is attended by an undue friability of the organ; often, too, the lobules are much less distinct than in the normal liver. In fifty-two cases, the fat was slight in amount, and twice it could not be demonstrated. These fifty-four cases are open to the interpretation that the fat is merely the expression of low vitality of the cell which is in some way prevented from carrying out completely its metabolism.

The cell may be likened to a mill which, with the usual number of workers, can handle a certain quantity of raw material, transform it into the finished product, without storing any of it on the premises. Let the mill be only half efficient by reason of half its employees being idle, and half its raw material is stored up on the premises. Or if the mill is suddenly supplied by one and a half times its usual raw material, one half of its daily income in raw material will be stored up. Thus the cell appears encumbered by fat when its working efficiency is lowered or when its fat supply is abnormally increased. We do not know whether the fat is the real raw material,

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or is an imperfectly finished product, but we assume that fat microscopically shown as such in the cell is material which in the normal liver cell would not appear; in other words, the normal cell-processes are accomplished with soluble materials, and these are ordinarily not to be demonstrated.

For the demonstration of the various types of fatty deposit in the liver, we have found Sudan III to be the most serviceable stain. Other methods, including staining by Nile blue sulphate, were also used, but we experienced some difficulty in interpreting our results from these sections. Although one of us (Klotz) has shown that *in vitro* the neutral fats, the fatty acids and the fatty acid soaps do give certain differences of color, yet by the use of Sudan III here no differentiation can be made with certainty between the neutral fats, fatty acids or oleic acid soaps, more particularly as these substances occur in mixtures.

From our observations we have been able to distinguish four types of fatty deposits in the lobules of the liver. Although these four types are readily distinguished, and are quite characteristic in themselves, we are by no means convinced that they differ as definitely in origin. These four types of fat deposit in the liver are as follows: (1) fat deposits in which globules alone are present in the lobule; (2) fat deposits in which granules alone are present; (3) fat deposits in which globules and granules are found in the same lobule; (4) fat deposits in which granules and globules are found in the same cell.

In the above classification we are laying considerable stress upon the *appearance* of the fat deposit in the liver. We are fully aware that a classification determined upon the physical characters of a substance is insecure, yet, as we shall point out later, these physical differences are associated with fairly definite differential staining properties.

Globules of fat may be of various sizes, ranging from minute spherical bodies to large masses occupying almost the entire liver cell. In some cells fat droplets of the smallest size are seen. These droplets are commonly aggregated towards one side of the cell, leaving the rest of the cytoplasm fairly free from fat droplets. As they increase in size, the droplets become fewer in number and the

nucleus becomes slightly shifted to the cell wall, away from the fat deposit. This eccentric position of the nucleus is increased as the fatty materials accumulate until eventually the nucleus becomes flattened and elongated along one border of the cell. When smaller globules are present, the nucleus occupies a central position and shows no evidence of distortion. Gradually, however, when the fat droplets in the protoplasm coalesce, the nucleus is shifted eccentrically, and eventually occupies a position on the outer border of the large fat drop. The ectoplasm of the cell forms the boundary of the fat drop, while the protoplasm of the cell is reduced to a small area lying about the eccentric nucleus. Both the nucleus and the reduced protoplasm form a crescentic mass over the fat drop. It is, however, to be noted that the nucleus never becomes so compressed as to simulate that of the true fat cell. When thick sections are made, and the nucleus of the liver cell is observed in a vertical position, it has a round contour, and gives the impression that it is lying in the centre of the cell. When seen laterally, however, the nucleus appears oval with blunt and rounded ends.

Occasionally liver cells are completely distended with fat; the fat, however, is separated into several globules which have not coalesced. These globules seem to have between them a thin dividing membrane, which prevents their coalescence. When such cells are ruptured, the fatty contents flow together to form a single mass.

Granules, on the other hand, are readily distinguished from globules of the smallest size. In the case of globules, the intensity of the Sudan absorption increases with the size of the globule, the smallest globules appearing the lightest in color. Granules are uniformly of a brownish color, not unlike that seen in the granules of brown atrophy. The contour of the granules is moreover irregular, and commonly one can make out sharp corners and edges in their structure. Cells are frequently packed with these irregular granules, in which there appears to be no tendency to coalesce. The granules frequently take a concentric arrangement about the nucleus, but in other cases they are distributed diffusely, or in the periphery of the cell protoplasm. These granules we think to be evidence of degeneration.

In staining with Sudan III and hematoxylin, one can readily pick

out the areas occupied by fat and distinguish the zone in the lobule which is affected. In the cases in which there is diffuse globular infiltration of fat in the lobule, it is difficult to make out the individual liver cells, as the knife drags a considerable amount of fat which blurs the section. In these cases of diffuse infiltration each liver cell is distended with the semifluid fat. In the older specimens crystals sometimes appear in these fat globules, probably crystals of neutral fats.

To sum up this question, we admit at the outset that we have no power of differentiating with certainty neutral fats from fatty acids or soaps. It is notable that fat appears in two forms, granular and globular, differing in appearance and perhaps in nature. We are not able to say whether one kind of fat can take two forms, although we are disposed to think that it does not, and that the globular and granular forms of fat may well be different forms, for example, the globular being perhaps neutral fat and the granular being soaps or yet other combinations.

In the fourteen cases which are definitely pathological, large globules of fat were always present, small globules frequently (eight), and granules rarely (three). If our supposition that the granules are quasi-pathological, be a correct one, it is likely that a liver so damaged as to be loaded with fat globules, would be perverted in function so that many of its cells would show fat granules; but the absence of these granules in eleven of fourteen cases seems to indicate that the two conditions do not necessarily arise from the same cause, or under similar circumstances; or else that the liver cell degeneration has advanced so far that it has passed the stage in which granules are to be seen.

The Globular Form.—There is no reason to suppose that small and large globules are different, for every gradation of size can be found, and globules appear to have the power of fusing just as they do in fluid; nor is there any reason to suppose that they exist outside of the cells, until such time as having distended the cells, they appear to have burst these and to have fused with other globules in inter-cellular spaces. In the light of the views held to-day as to the nature of the cell wall, namely, that it is a colloidal concentration of protoplasm rather than a real membrane, we find it

hard to see why this so-called membrane should be resistant long after the cell protoplasm has been replaced: we incline to think that the signet-ring cell may gradually merge into a mere mass of globular fat, the nucleus disappearing. The most potent argument for this view is the huge size of fat globules in the liver. A digression may be made here to remark that in the case of globules of fat, large but not larger than the normal cell, the existence of this fat is consistent with the life and usefulness of liver cells. Whether the signet-ring cells functionate as liver cells we cannot say, but liver function can apparently be performed when no normal liver protoplasm other than nuclear can be made out in the section. It is well to make a mental correction of the apparent functional power of fatty liver tissue, because the tissue probably always seems more injured than it really is.

The fourteen cases of intense fatty change in this series do not arrange themselves naturally: twice they are alcoholic (a condition in which intense fattiness is well known to be almost, if not quite universal), seven times septic, five times toxic as the result of chemical poisons (compare the well-known effect of phosphorus), and once uremic. The last named was in no sense septic, and suggests that sepsis is not a necessary cause of this state: yet the fourteen cases uniformly exhibit a metabolic disturbance caused by a chemical or otherwise toxic agent. This observation does little more than support our previous supposition that fat is an evidence of the inability of the liver to perform satisfactorily one of its perhaps lesser functions, viz., the metabolism of fat. Carrying this statement further, we would expect to find that the mere process of disease-produced death, would, in itself, be attended by a lowering of metabolic power, so that all livers, save those found after instant death by accident, might be expected to show some fat; and so it appears to be.

Distribution of Large Globules.—Large globules occur most frequently in all parts of the lobules (twenty-seven): when one part only of the whole lobule is affected it is oftenest the peripheral (twelve), next oftenest the central (six), the middle zone never being the only part affected. If we count all combinations, globules are peripheral in forty-six, central in forty, middle in thirty-one. No generalization from this statement is possible, unless it may be said

that the part which has the best double blood supply, viz., the mid-zone, is least often affected, and the change is not definitely connected with an over-supply of fat from the portal blood, otherwise the mid-zone would be more liable than the central. More likely does it seem that the mid-zone, which has, in addition to its portal supply, a supply of oxygenated blood from the hepatic artery, is by reason of its better supply of oxygen able to use up any excess of fat. Further, the central zone being next in order to receive oxygenated blood, is freer from this form of fat than the peripheral zone, which has only its portal supply.

Granular fat, on the other hand, we suppose indicates degeneration of the protoplasm, a condition that is not bettered by an increased oxygen-supply. Nor was the chronic passive congestion of the nutmeg liver a predisposing factor in those cases where the central zone was much affected; this observation at first sight appears to conflict with what we have just stated, because stagnated venous blood is the least oxygenated of all, but our supposition is not that lack of oxygen caused the defect, but that plentiful oxygen remedies it.

Irregular Areas of Large Globules.—In one case, groups of large globules appeared quite at random. It must be kept in mind that the knife may carry isolated globules across the section into different parts of the tissues, but this is generally readily recognizable. Twice, however, we have found a remarkable state of affairs: a definitely bounded macroscopically yellow area has proved to be made up of huge globules, the mass looking microscopically like a lipoma; however, in the liver tissue nearby globules of various size in the cells have indicated that the condition was one of gradual cellular acquirement, although we have no idea of the nature of the process which permits such a complete metamorphosis of localized pieces of tissue. These masses comprised many lobules, and their boundaries were those of lobules, a part of a lobule not entering into this state while the rest remained healthy.

Granular Fat.—Granular fat occurred more often than the globular, in the proportion of eighty-seven to fifty-eight. Granular fat alone occurred in forty, globular alone in eleven, while both were combined in forty-seven instances. The usual form is a slight dis-

tribution of granular fat throughout the lobules, while with this change are often united various distributions of globules. This universally distributed granular fat we suppose to be the true fatty degeneration of the liver (the word being used to designate a condition comparable to that found in the heart, and without prejudice to the question whether it is ultimately true infiltration or protoplasmic transformation). Whether these granules are a special form of fat, or whether they can coalesce and form globules we cannot say, but we think that they cannot coalesce. The occurrence of such universal granular fat appears to be consistent with otherwise perfectly healthy liver tissue, judged by the microscopic appearance, with ordinary stains.

When the granules are not diffuse but are limited to one or more zones of the lobule, they are most often central (ten), less often peripheral (two), and never median. If these granules have to do with ill nutrition or slight intoxication, as we suppose, it is to be expected that the part of the lobule doubly supplied with blood should be most free from them.

The appearance of fat when it occurs in the granular form is so distinct from the globular type that there is no difficulty in recognizing the condition. The character of the granules, whether in the peripheral or central zone, is the same. The granules first appear as very minute points distributed in the protoplasm of the liver cell without affecting the position of the nucleus. The granules later increase in size and also in number, until the cytoplasm is loaded with these discrete bodies, which have little if any tendency to coalesce. In no instance was it found that the quantity of fatty granules altered the central position of the nucleus, nor was the degeneration associated with any nuclear change.

Fat Globules and Granules in the Same Lobule.—In a fair number of cases it was noted that in the same liver lobule there appeared a deposit of fat, both of the granular and globular type. When these deposits were thus associated, the granular deposit was present in a diffuse form, while the fat globules were most commonly found in the peripheral zone. Such cases we describe as fat distributed diffusely in the lobule. The deposit of granular fat in the periphery was similar to the deposits in which globules alone were present in

the lobule, and we could make out no direct association between the deposit of the globular and granular forms.

In other instances it was found that the granular deposit was predominant in the lobule, and only here and there was a cell or a small collection of cells containing fat globules. In these cases the globular deposit appeared to select no particular zone, but occurred sporadically in the lobule. These globular deposits were often very few, only one cell in a lobule, which was otherwise crowded with granular fat showing this deposit.

It is probable that, where there is a considerable deposit of fat in one or other zone of the liver along with the granular deposit in the other zones, we have to do with two separate pathological conditions, the one being superadded upon the other.

Granules and Globules of Fat Appearing in the Same Cell.—As we have noted above, fat may appear in the granular and globular type in the same lobule, and in some instances it appeared as if some of the granular deposit had coalesced to form globules. In only a few instances was it noted that definite small globules were present in a cell, while granules were also to be noted in the protoplasm of the same cell. This appearance was found the least frequently. It was sometimes noted that the globules in these cells had the same dark staining characters as were seen in the granular fat deposit. These cells containing both granules and globules had no fixed situation in the liver lobule, but were to be found in any of the three zones.

Fat in the Central Zone.—Our sections show that when we are considering the central zone alone, the fat found here is oftener granular (thirty-two), less often globular (sixteen). If our supposition that globular fat is a "physiological" phenomenon be a correct one, the difficulty is not to see why globular fat should be the rarer form in the central zone, but why it should be there at all, especially when the mid-zone is repeatedly free from it; yet we found this condition in six cases.

Is there a deposition of fat characteristic of uremia? In thirteen cases of uremia the fat was extreme once, considerable nine times, slight three times; it was diffuse four times, and the single areas affected were central (nine), peripheral (seven), middle (six); five

times granular, four times granular and globular, and three times globular. From this observation, we are compelled to think that there is not a characteristic distribution of fat in cases of uremia.

It is of interest to note that of the sixteen cases in which the fat appeared in the central zone alone, thirteen were in females. Five of these were associated with recent pregnancies, three with uremia, one with pus tubes, and four with various infections.

CONCLUSIONS.

1. Nearly all livers at autopsy contain fat which is histologically demonstrable.

2. Fatty substances in the liver appear chiefly in two forms, as small granules and as globules of various sizes.

3. We suppose that granules indicate the (protoplasmic) change commonly spoken of as fatty degeneration, and that globules of small size are excess of fat, stored up by reason of some pathological change which may be merely temporary.

4. Fat is oftenest central, least often in the mid-zone.

5. A heavy deposit of fat is compatible with a competent liver.

6. Intense fattiness, generally globular, occurs with intoxications of bacterial and chemical nature, as well as in cases where a complex toxin is manufactured by the body-cells.

7. Granular fat occurs oftener than globular: it affects most often the central zone; globular affects most often the peripheral.

8. "Accidental" masses of globular fat are found at times, and appear to follow no rule of position: these are comparable to lipomata, which are evidently the result of a pathological process.

9. We found no deposit of fat characteristic of uremia.

10. We know of no analysis of the liver fats in the granular and globular states respectively: it seems to have been taken for granted that the deposits were one and the same. To determine the relationship, if any, between these two forms should be the basis of investigation.

OBSERVATIONS UPON THE ACTION OF SALTS ON THE CATALASE OF THE BLOOD IN RABBITS.[1]

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The action of salts upon organic catalase has been studied in considerable detail by various observers (Kastle and Loevenhart, Jacobson, Issajew, Senter, etc.) and will, therefore, be dismissed without further comment. The object of this work was to study the action of substances upon the catalytic activity of the blood in the animal body, and although the results are far from complete, it will be seen that it is quite impossible to draw conclusions concerning the action of substances on catalase in the body from test-tube experiments.

The following subjects will be briefly presented:

1. The effect of the feeding of sodium bicarbonate upon the catalytic activity of the blood.
   (A) Toxic action of large amounts of sodium bicarbonate upon the kidneys.
2. The subcutaneous injection of sodium bicarbonate.
3. Intravenous injection.
   (A) Distilled water.
   (B) Salts, Acids and Alkalies.
4. Inhalation.
   (A) Carbon dioxide.
   (B) Illuminating gas.

THE EFFECT OF THE FEEDING OF SODIUM BICARBONATE UPON THE CATALYTIC ACTIVITY OF THE BLOOD.

Sodium bicarbonate was arbitrarily chosen as the salt to be fed. In all of these experiments rabbits were used. The salt was dissolved in water and administered by stomach tube in one, two, four and ten gram doses. Briefly, this had no effect on the catalytic

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activity of the blood even when administered every day for several weeks.

On the other hand, these experiments were complicated by the action of the salt upon the kidneys. In every instance albumen and casts followed its administration, and while, in the majority of cases, the albumen was found only in traces and the casts as occasional hyaline forms, in several animals there was total suppression of urine followed by convulsions and death. Two such instances occurred among the first feeding experiments, and, singularly, one animal was pregnant, and the other, during the feeding and before the urinary changes manifested themselves, gave birth to four young. In still another case the animal aborted and then developed uraemia. The kidneys in these fatal cases were swollen, gray or mottled and showed small, pin-point hemorrhages on the surface. Microscopically the epithelium of the tubules was swollen, vacuolar and often desquamated, and casts occurred in many of the collecting tubules. It is of interest to note that albuminuria and casts have been reported in the urine of rabbits following the administration of sodium chloride (Leopold).

The catalytic activity of the blood in these fatal cases declined rapidly and presented the same curve described in the production of acute nephritis with uranium nitrate (Winternitz).

THE INFLUENCE OF SUBCUTANEOUS INJECTIONS OF SALTS UPON THE CATALYTIC ACTIVITY OF THE BLOOD.

Jolles reports an isolated experiment where, by injecting normal hydrochloric acid into the peritoneal cavity, it was hoped to lower the alkalinity of the blood and thereby decrease its catalytic action. The animal died and a decrease in the catalytic activity of the heart's blood was found. However, it is not stated how soon after the injection the animal died, and this, as will be seen, is an important factor.

The subcutaneous method is ill adapted for this work on account of the slow absorption of the fluid and was, therefore, abandoned for intravenous injections, which, as will be seen, offered a ready means of study.

THE INFLUENCE OF INTRAVENOUS INJECTIONS OF SUBSTANCES UPON THE CATALYTIC ACTIVITY OF THE BLOOD.

Method.—A number of experiments were tried before a satisfactory method of testing the effect of salts by intravenous injection was found. Bicarbonate of soda was the salt used in these first experiments, and we found that either there was no decrease in the catalytic activity of the blood taken at leisure after relatively large injections of the salt, or that the animal died and showed a marked decrease in the power of the heart's blood to split hydrogen peroxide. These difficulties, however, were overcome by the following method: the animal was anesthetized, the femoral vein exposed and a canula, connected with a burette, inserted. The salt was placed in the burette, kept warm by a coil. The animal was bled just before the intravenous injection was started, at intervals during the injection, immediately preceding its cessation, and again at one and at five minutes after its cessation. The activity of the blood was tested by the method previously described (Winternitz).

(*A*) *Distilled Water.*—The effects of intravenous injection of distilled water will first be recorded in order to estimate the action of the simple diluting effect of the blood on its power of decomposing hydrogen peroxide.

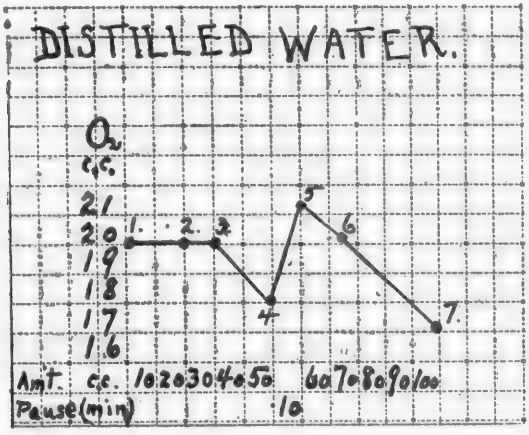

CHART I.

Test.	Remarks.
1. Before injection.	Injection started.
2. After injection of 10 to 20 c.c.	
3. After injection of 30 to 40 c.c.	
4. After injection of 50 c.c.	Pause of 8 minutes.
5. After pause of 8 minutes.	
6. After injection of 50 to 75 c.c.	
7. After injection of 75 to 100 c.c.	

The above curve shows that after the injection of fifty cubic centimeters of water there is a very slight decrease in the catalytic activity of the blood (two cubic centimeters), which was gradually compensated, so that eight minutes after the infusion was discontinued the action was again normal, while with one hundred cubic centimeters there was a more distinct fall.

In using solutions of salts, therefore, any marked variation in the catalytic action of the blood may be attributed to the action of the salt, since the diluting action of the fluid has only a slight effect upon the power of the blood to split hydrogen peroxide.

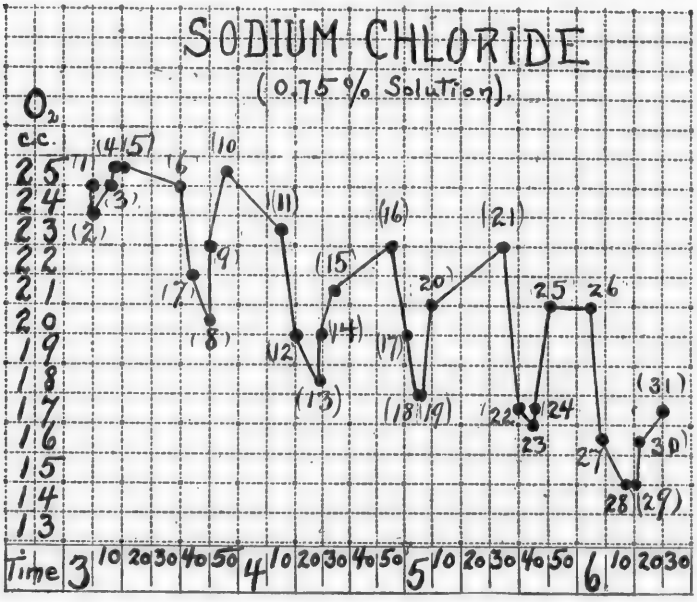

CHART 2.

(*B*) *Salts. Sodium Chloride.*—In the first experiment to be recorded, an isotonic solution was used (0.75 per cent.). The curve given above illustrates the effect.

Test.	Time.	Remarks.
1. Before injection.	3:05 p.m.	Injection started.
2. After 10 c.c.	3:06	
3. After 25 c.c.	3:11	Injection stopped.
4. After 1 minute pause.	3:12	
5. After 6 minute pause.	3:17	
6. After 24 minute pause.	3:35	Injection started 3:36 p.m.
7. After 10 c.c.	3:39	
8. After 25 c.c.	3:45	Injection stopped.
9. After 1 minute pause.	3:46	
10. After 6 minute pause.	3:57	
11. After 25 minute pause.	4:10	Injection started 4:11 p.m.
12. After 10 c.c.	4:16	
13. After 25 c.c.	4:24	Injection stopped.
14. After 1 minute pause.	4:25	
15. After 6 minute pause.	4:30	
16. After 26 minute pause.	4:30	
17. After 10 c.c.	4:50	Injection started 4:55 p.m.
18. After 25 c.c.	4:57	
19. After 1 minute pause.	5:01	
20. After 5 minute pause.	5:02	
21. After 19 minute pause.	5:06	
22. After 10 c.c.	5:30	Injection started 5:35 p.m.
23. After 25 c.c.	5:36	
24. After 1 minute pause.	5:40	
25. After 1 minute pause.	5:41	
26. After 20 minute pause.	5:47	
27. After 10 c.c.	6:00	Injection started 6:01 p.m.
28. After 25 c.c.	6:04	
29. After 150 c.c.	6:12	Injection stopped.
After 1 minute pause.	6:16	
After 9 minute pause.	6:17	
	6:25	

The curve illustrates that sodium chloride in isotonic solution has a transient inhibitory action upon the catalytic activity of the blood. After twenty-five cubic centimeters in this experiment there was very little drop, and this was rapidly recovered from. After fifty cubic centimeters the drop was much more marked but it was completely compensated within five minutes. After seventy-five cubic centimeters the drop was still more definite and the com-

pensation was not complete even after a pause of fourteen minutes. It is evident, therefore, that the tissues of the body are able to counteract in some way the inhibiting effect of sodium chloride, but that this compensation gradually becomes less efficient. This is evidenced by the greater decrease in the action of the blood on hydrogen peroxide following each injection and in the prolonged times necessary for the compensation which gradually becomes less and less complete.

In another experiment a 5 per cent. solution of sodium chloride was used.

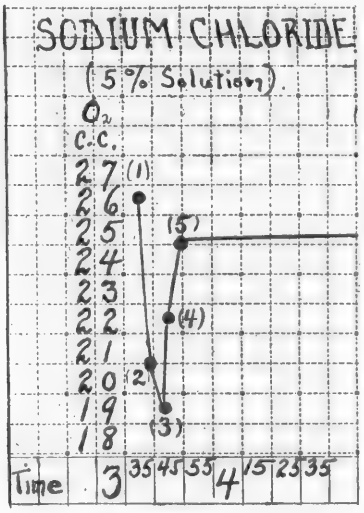

CHART 3.

Test.	Time	Remarks.
1. Before injection.	3:35 p.m.	Injection started 3:36 p.m.
2. After 10 c.c.	3:38	
3. After 25 c.c.	3:40	Injection stopped.
4. After 1 minute pause.	3:45	
5. After 5 minute pause.	3:49	
6. 1 hour pause.	4:44	Injection started.

The result of one injection of twenty-five cubic centimeters will suffice to show that the concentrated solution of sodium chloride

has a much greater inhibiting effect upon the catalase of the blood than the normal solution.

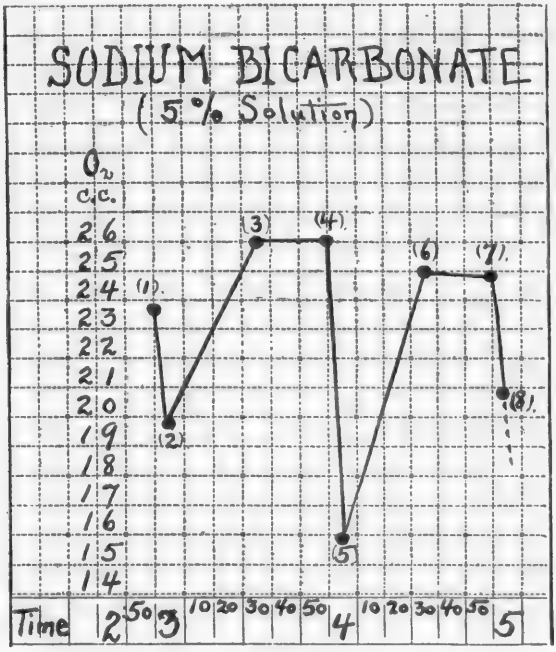

CHART 4.

Test.	Time.	Remarks.
1. Before injection.	2:55 p.m.	Injection started.
2. After 20 c.c.	3:00	Injection stopped. Dyspnea, etc.
3. After 30 minute pause.	3:30	
4. After 55 minute pause.	3:55	Started injection 3:55+.
5. After 6 c.c.	4:00	Dyspnea, etc., as above.
6. After 30 minute pause.	4:30	Infusion stopped.
7. After 55 minute pause.	4:55	Injection started 4:55+.
8. After 3.5 c.c.	5:00	Dyspnea, convulsions, opisthotonous; death; heart blood.

The action of bicarbonate of soda is analogous to that of sodium chloride. It is of interest to note, however, that only six cubic

centimeters of solution in the second injection brought about a much greater inhibition of the catalytic activity of the blood than twenty cubic centimeters in the first, and that three and one-half cubic centimeters in the third injection were fatal.

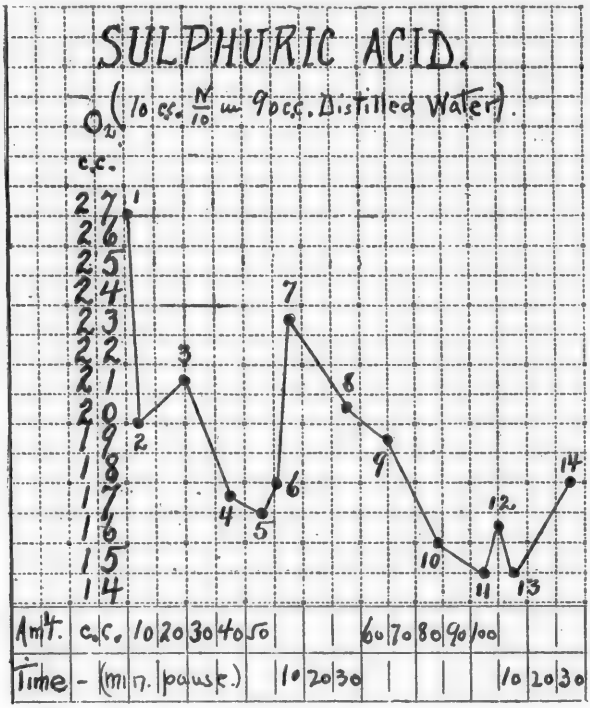

CHART 5.

Sulphuric Acid.

Test.	Remarks.
1. Before injection.	Injection started.
2. After 5 to 10 c.c.	
3. After 20 to 30 c.c.	
4. After 32 to 45 c.c.	
5. After 50 c.c.	Injection stopped.
6. After 1 minute pause.	
7. After 10 minute pause.	

Test.	Remarks.
8. After 30 minute pause.	Injection II started.
9. After 10 to 20 c.c.	
10. After 25 to 35 c.c.	
11. After 50 c.c.	Injection II stopped.
12. After 1 minute pause.	
13. After 5 minute pause.	
14. After 30 minute pause.	

The only comment necessary here is the lack of compensation after the injection was discontinued.

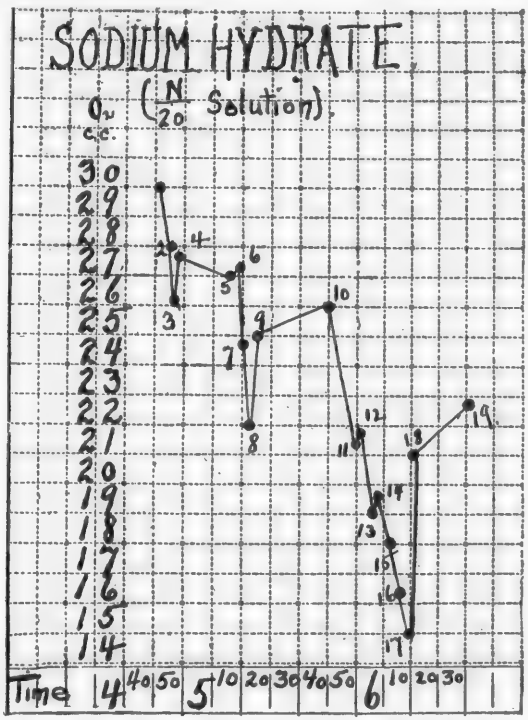

CHART 6.

Sodium Hydrate N/20 Solution.

Test.	Time.	Remarks.
1. Before injection.	4:15 p.m.	Injection started 4:49 p.m.
2. After 10 to 15 c.c.	4:50	

Test.	Time.	Remarks.
3. After 15 to 20 c.c.	4:51	Injection stopped.
4. After 1 minute pause.	4:52	
5. After 19 minute pause.	5:10	Injection II started.
6. After 30 c.c.		
7. After 40 c.c.		
8. After 50 c.c.	5:18	Injection II stopped.
9. After 2 minute pause.	5:20	
10. After 25 minute pause.	5:45	Injection III started.
11. After 10 c.c.		
12. After 20 c.c.		
13. After 40 c.c.		
14. After 50 c.c.		
15. After 70 c.c.		
16. After 90 c.c.		
17. After 100 c.c.		
18. After 1 minute pause.		
19. After 22 minute pause.		

The last two curves given above are similar in every respect to those representing the injection of sodium chloride, etc., and, therefore, are self-explanatory. It is, however, noteworthy that both of these animals lived and apparently suffered no ill effects from the introduction of acid or alkali into their circulation.

Sodium Sulphate and Potassium Iodide.—Several salts, including potassium iodide and sodium sulphate, were shown by Kastle and Loevenhart to have an accelerating effect upon the catalase of an extract of pig's liver, and for this reason some of these salts were chosen in order to see if a similar effect could be produced in the blood of a living animal. Sodium sulphate and potassium iodide in 0.75 per cent. strength were used and it may be said that even in this dilute form, ten cubic centimeters of the solution of either salt has a definite though slight inhibiting action upon the power of the blood to split hydrogen peroxide.

INHALATION.

Two gases were tried, carbon dioxide and the carbon monoxide present in illuminating gas. The first has absolutely no effect even when administered until convulsions are produced. With the second, however, there is a definite decline in the catalytic activity of the blood.

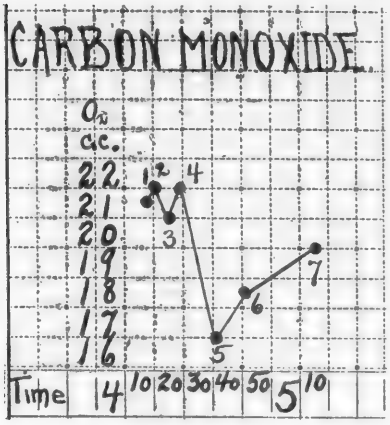

CHART 7.

Test.	Time.	Remarks.
1. Before inhalation.	4:12 p.m.	Inhalation begun 4:13 p.m.
2. Polypnea.	4:16	
3. Convulsions.	4:20	Inhalation discontinued.
4.	4:23	
5.	4:37	
6.	4:47	
7.	5:10	

The above curve shows a decided fall in the catalytic activity of the blood following the administration of illuminating gas. This fall, however, does not manifest itself during the inhalation, but only after the convulsions appear and the inhalation is discontinued. And it differs from the fall resulting from the injection of salts in a second respect, inasmuch as it is only gradually compensated. It might be remarked that this experiment had been previously tried by Jolles, who found, however, that illuminating gas administered to animals even in fatal amounts had no effect on the catalytic action of the blood. We are unable to explain this discrepancy in the results except perhaps that there might be a difference in the gas used. In the experiment recorded the gas was obtained directly from the city supply and allowed to bubble through water before administration.

SUMMARY.

Bicarbonate of soda fed in varying quantities over a period of months has no direct effect upon the catalytic activity of the blood. When fed to rabbits, it invariably causes a slight albuminuria and occasional casts. The kidneys of pregnant animals, especially in the later stages of pregnancy, seem to be more susceptible to this salt, and in some instances there may be complete suppression of urine followed by death. In these cases there is a decline in the catalytic activity of the blood similar to that observed in cases of acute nephritis, following the administration of uranium nitrate.

The subcutaneous injection of salts is impracticable on account of the slow absorption.

In intravenous injections it is important to make observations during the injection and at short intervals after the injection has been discontinued, since there is a rapid compensation on the part of the organism with a return of the normal catalytic activity of the blood.

The simple diluting effect brought about by the injection of distilled water into the circulation manifests itself as a slight transitory decrease in the catalytic activity of the blood. This must be taken into consideration when estimating the effect of the injection of solutions of salt upon the catalase of the blood.

Salts, such as sodium chloride, sodium bicarbonate, sodium sulphate and potassium iodide, acids, such as sulphuric, and alkalies, such as sodium hydrate, cause a definite decrease in the catalytic activity of the blood. This decrease varies directly with the concentration of the salt. The organism compensates rapidly and brings about a return to the normal activity. This compensation becomes less and less complete as larger quantities or repeated infusions are made. For example, twenty cubic centimeters of a five per cent. bicarbonate of soda solution causes a marked fall in the catalytic action of the blood. This is rapidly recovered from in from one to five minutes, and then a much more marked fall in the activity, requiring a longer time for compensation, occurs after the injection of six cubic centimeters of the same solution.

It is of interest to note that sodium sulphate and potassium iodide, which have been shown to accelerate the catalytic activity of liver

extract in the test-tube, have an inhibiting effect on the action of the blood when injected intravenously. This is only another instance of the dangers to be encountered from the immediate application of test-tube experiments to the living organism.

The inhalation of carbon dioxide gas, even until convulsions are manifested, is without effect on the catalytic activity of the blood. On the other hand, there is a definite decline in this activity following the administration of illuminating gas. In this respect our results are directly at variance with those of Jolles.

HYPOTHESIS.

As has been previously shown, there is a decline in the catalytic activity of the blood when there is a functional insufficiency of the kidneys in rabbits (Winternitz). At the beginning of this work it was hoped to bring about a more or less analogous condition by the action of salts. In nephritis, as is well known, there is a salt retention and it seemed that here might be the explanation of the decline in the catalytic activity of the blood accompanying imperfect or suppressed renal function. The above experiments seem to bear out this theory. It will be remembered that there is a decline in the catalytic activity of the blood following the intravenous injection of salts. The decline is only transitory and is followed by a rapid compensation, but with each subsequent injection there is a greater decline in the activity followed by a longer compensation time. At length the tissues no longer compensate and a fatal result is produced. It might be argued that this is somewhat analogous to what occurs in nephritis. For a time the tissues of the body will take care of products which normally should be eliminated by the kidneys. During this period of compensation there will be no decrease in the catalytic activity of the blood, but as soon as the tissues fail to compensate there will be a decline in this action accompanying the uraemic symptoms. If the tissues are still able to compensate, then the activity will return to normal with the disappearance of the uraemic symptoms; but if the tissues are unable to compensate, then the decline in the catalytic activity of the blood will be continued and the uremia will end fatally. Uremia would then appear to be an embarrassment of the "factors of safety" brought about primarily by renal insufficiency.

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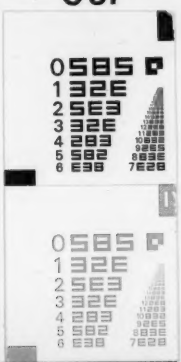
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